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Estimation of individual relative risk of malignant neoplasm using cytogenetic examination data of the Chernobyl accident clean up workers

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Summary

The **purpose** of this work was to differentiate groups of Chernobyl clean up workers with elevated individual relative risk (IRR) of tumor development on the basis of lymphocyte cytogenetic changes considered as cancerogenic factors.
Materials and methods: A cohort of 2076 liquidators who worked in the accident zone in 1986-1987 was examined for cytogenetic changes in the irradiated lymphocytes, cultured using a standard technique. All kinds of chromosome aberrations accessible with the traditional chromosome-painting method were determined.
Results: The risk of development of malignant neoplasm in the cohort investigated was calculated for 155 persons, i.e. their prevalence being 7,47%. The frequency of chromosomal aberrations has the lowest value of self-descriptiveness at cohort stratification in groups with malignant and in those without it, and the frequency of pair fragments and chromatid aberrations has the highest value. The calculation of IRR has revealed the existence of groups with different risk level among 337 persons without pathology at the time of cytogenetic examination. Only 45,1% of persons had their IRR close to the value of the relative risk of development of malignant neoplasm. In comparison with the mean value for the population of Ukraine, IRR changed from 5 to 45 and for 75% of persons from cohort it did not exceed 20.
Conclusions: The method of evaluation presented in this work shows that cytogenetic examination is an important part of monitoring of the Chernobyl clean-up workers which makes it possible to reveal groups with different risk levels among persons without malignant neoplasm at the time of investigation.
Key words: clean-up workers, individual relative risk, malignant neoplasm, individual radiosensitivity, cytogenetic parameters.

Oszacowanie osobniczego ryzyka względnego zachorowania na nowotwór złośliwy przy zastosowaniu badania cytogenetycznego u służb porządkowych po katastrofie w Czernobylu

Streszczenie

Celem pracy było wykazanie istnienia grup pracowników usuwających skażenia po awarii reaktora w Czarnobylu, u których można było stwierdzić podwyższony poziom względnego ryzyka (IRR) wyindukowania nowotworów złośliwych na podstawie aberacji chromosomowych w limfocytach jako czynnika kancerogennego.
Materiał i metoda: Grupa badana składała się z 2076 osób, pracujących przy usuwaniu skażeń powstałych na obszarze awarii w latach 1986-87. Grupę przebadano metodami standardowymi pod kątem zmian cytogenetycznych w limfocytach naświetlonych w wyniku awarii, które związane były z nieprawidłowościami w składzie chromosomalnym stosując dostępne techniki barwienia chromosomowego.
Wyniki: Występowanie złośliwych nowotworów w badanej grupie wykryto u 155 osób, tzn. w 7.47% przypadków. Częstość występowania nieprawidłowości chromosomalnych przyjmuje najmniejszą wartość tzw. wskaźnika „self-descriptiveness" w grupach charakteryzujących się patologią nowotworową lub bez tej patologii, natomiast najwyższą wartość, w zakresie częstości występowania fragmentów par i nieregularności chromatycznych. Zależnie od wartości IRR wśród 337 osób bez patologii podczas badania cytogenicznego określono istnienie grup charakteryzujących się różnym stopniem ryzyka. Jedynie w przypadku 45.1% osób wartości IRR były zbliżone do wartości względnego prawdopodobieństwa występowania nowotworu złośliwego. W porównaniu ze średnią dla całej populacji Ukrainy wartości IRR wahały się w granicach od 5 do 45, zaś u 75% grupy badanej nie przewyższały one 20.
Wnioski: Metoda oceny przyjęta w niniejszej pracy wykazuje, że badanie cytogenetyczne stanowi ważny składnik procesu monitorowania stanu zdrowia pracowników usuwających skażenia po awarii w Czarnobylu. Pozwala ona również w rozsądny sposób wyodrębnić grupy pracowników o różnym stopniu ryzyka, charakteryzujących się patologią nowotworową lub bez tej patologii w czasie przeprowadzania badań.
Słowa kluczowe: służby porządkowe, względne ryzyko zachorowania, nowotwór złośliwy, osobnicza promieniowrażliwość, parametry cytogenetyczne.

Cytogenetic estimation of the risk of a malignant neoplasm to be developed in an individual makes it possible to reveal groups with different risk levels among the persons without the disease at the time of the examination.

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Introduction

Taking into account the global character of the effect of Chernobyl accident on the population it is clear that it has received the status of a "Chernobyl catastrophe". About 8% of the population suffered from the Chernobyl accident in Ukraine, including 350 thousand clean-up workers requiring immediate medical supervision [1]. The doses received by clean-up workers over a relatively short time of 1-2 months, basically exceeded the predicted lifelong doses for the population living in radiation-polluted territories. Consequently, the dynamics of the manifestation of the stochastic effects of radiation for a clean-up worker cohort can be more intensive than that for the inhabitants of the radiation-polluted area [2]. The increase in the number of cases of malignant neoplasm in clean-up worker cohorts, the part of which can be radiation-induced, is of certain interest [3,4]. Besides the literature data which testify to the increased level of radiation-induced changes in clean-up workers, cytogenetic status as well as radiation-induced genome instability can underlie cancerogenic effects [5,6].

The purpose of this work is to prove the existence of specific groups of clean-up workers with an elevated individual relative risk (IRR) of malignant neoplasm on the basis of cytogenetic changes studies in the peripheral blood lymphocytes, considered as cancerogenic factors.

Material and methods

A cohort of 2076 clean-up workers was investigated. They received documented doses of radiation when they worked in the accident zone in 1986-87 and were referred to the Kiev Regional Chernobyl Expert Council in 1990-1996 with the aim of establishing a correlation between their diseases and their work during the elimination of consequences of the accident.

The diagnosis of a "malignant neoplasm" was verified morphologically in all cases.

Human-irradiated peripheral blood lymphocytes cultivated with a standard technique were subjected to cytogenetic investigations. All kinds of chromosome aberrations accessible for a conventional chromosome staining analysis were registered. Three hundred metaphases for every surveyed person were analyzed on average.

The following chromosome parameters were registered: frequency of damaged cells (x_1), frequency of aberrations of chromosomes (x_2), frequency of chromatid aberrations (x_3), frequency of chromosomal aberrations (x_4), frequency of pair fragments (x_5), frequency of acentric rings (x_6), frequency of centric rings (x_7), frequency of dicentrics (x_8), and frequency of translocations (x_9).

The calculation of relative risk of the disease is:

$$RR = \frac{v_1}{v_0},$$

where v_0 - is the disease frequency in non-exposed group and v_1 - is the frequency of the disease in the exposed group used in a standard approach for cohort research [7]. In our study, the population of Ukraine was considered as a non-exposed group, a cohort of clean-up workers was treated as an exposed group, and malignant neoplasm as examined disease. Calculation of relative risk *(RR)* was made for group and it does not take into account the individual parameters of exposed persons.

For IRR estimation we stratified the cohort for exposed persons with malignant neoplasm (group X) and for those without malignant neoplasm (group Y), then we determined the affinity measure (degree of similarity) of cytogenetic parameters in group Y with the same parameters of exposed persons in group X. As a working hypothesis it was accepted that the absolute individual risk (AIR) is directly proportional to the proximity measure of individual parameters to parameters of persons from group X.

We designated k - as a number of persons in cohort, n - as a number of persons in group X, m - as a number of persons in group Y ($m = k - n$), v - as the disease frequency in the cohort. Each individual from cohort was put in conformity with the vector of features $(x_1,x_2,...,x_l)$. Then for each of the parameters x_i, $i = 1,...,l$ in groups X and Y we calculated confidential intervals $[\alpha_i,\beta_i]$, $[\alpha_i^*,\beta_i^*]$, $i = 1,2,...,l$ with a given significance level α and lengths $a_i = \beta_i - \alpha_i$, $b_i = \alpha_i^* - \beta_i^*$, respectively, the crossing of length being c_i. The degree of self-descriptiveness of the parameter x_i, $i = 1,...,l$ was introduced as

$$I_i(X,Y) = 1 - \frac{c_i}{a_i + b_i - c_i} \qquad (1)$$

The parameters which confidential intervals are not crossed ($I_i = 1$), have the maximal value of self-descriptiveness and the parameters for which the confidential intervals coincide ($I_i = 0$) are minimal. In other cases $0 < I_i < 1$.

The measure of individual affinity P to X is determined by the formula

$$\rho(P,X) = \frac{\sum_{i=1}^{l} I_i(X,Y)\,\delta(x_i)}{\sum_{i=1}^{l} I_i(X,Y)}, \quad \text{where} \quad \delta(x_i) = \begin{cases} 1, & x_i = \in [\alpha_i,\beta_i], \\ 0, & x_i = \notin [\alpha_i,\beta_i]. \end{cases} \qquad (2)$$

For the group with the disease $\delta(x_i)$ is always 1, so $\rho(P,X)=1$. If any parameter from the individual feature vector P does not coincide with the correspondent confidential interval constructed in group X, then $\rho(P,X)=0$. In other cases $0 < \rho(P,X) < 1$. The degree of self- descriptiveness the parameters $I_i(X,Y)$ in formula (2) are the weight factors describing the importance of the parameter x_i.

To calculate the absolute individual risk $p_x(P)$ of an individual malignant neoplasm P from a cohort we apply the linear interpolation, then

$$p_x(P) = (1 - \alpha)\,((1 - v)\,\rho(P,X) + v)$$

The type of the interpolation formula should be specified using an analysis of distribution of disease frequency in groups with a different rates of affinity to group X. The final type of dependence (3) can be established only in a sufficiently long time cohort research.

Results

Malignant neoplasm in the cohort investigated was determined in 155 persons; their prevalence in the cohort was 7.47%. The average malignant neoplasm prevalence in Ukraine in 1994-1996 was 1.42% [9]. Thus, the malignant neoplasm relative risk in the clean-up workers cohort is 5.25.

Table 1 shows cytogenetic indexes of self-descriptiveness that were calculated from formula (1) and were determined on the basis of the examination of clean-up workers. It can be seen that the frequency of chromosomal aberrations has the lowest value of self descriptiveness in cohort stratification in groups of persons with malignant neoplasm and those without them, and the frequency of pair fragments and chromatid aberrations has the highest value.

It is well known that the risk is the probability of the development of an adverse event. In our research, the probability of the development of malignant neoplasm has been considered. It is necessary to note that the presence of a high risk factor does not mean that the person will definitely become ill. Even if the prognosis inaccurate at an individual level, it is certain in a large number of similar cases. Thus the risk factor can be an indirect marker of increased probability rather than the cause of similar development of the disease.

The application of the above approach in the calculation of individual risk made it possible to reveal groups with different risk levels among 337 persons without malignant neoplasm at the time of cytogenetic examination *(Table 2)*.

As we can see, only 45.1% of persons have their IRR values close to the malignant neoplasm relative risk in the cohort (from n to 2n); for others it changes over a wide range. Accordingly, IRR in comparison with the population of Ukraine changes from 5 to 45, and for 75% persons from the cohort it does not exceed 20.

It is necessary to note that for a more detailed estimation of the risk of malignancy the application of a complex genetic stability of somatic cells is necessary. It can result

Table 1. Self-descriptiveness of cytogenetic indexes.

i	α_i	β_i	α_i^*	β_i^*	a_i	b_i	c_i	I_i
1	5.563	9.847	6.502	7.741	4.284	1.239	1.239	0.711
2	4.956	9.507	6.503	7.941	4.551	1.438	1.438	0.684
3	1.432	4.673	2.517	2.993	3.241	0.476	0.476	0.853
4	3.695	5.610	3.948	5.302	1.915	1.354	1.354	0.293
5	1.918	3.830	1.562	2.023	1.912	0.461	0.105	0.954
6	0.000	1.091	0.631	0.971	1.091	0.340	0.340	0.688
7	0.000	0.372	0.225	0.360	0.372	0.135	0.135	0.637
8	0.409	1.064	0.752	1.449	0.655	0.697	0.312	0.700
9	0.339	1.387	0.654	0.904	1.048	0.250	0.250	0.761

Table 2. Evaluation of relative individual risk for clean-up workers.

In cohort			Compared with the population of Ukraine		
Risk group	**Quantity**	**%%**	**Risk group**	**Quantity**	**%%**
$v \div 2v$	152	45.1	$5v_0 \div 10v_0$	152	45.1
$2v \div 3v$	18	5.3	$10v_0 \div 15v_0$	18	5.3
$3v \div 4v$	89	26.4	$15v_0 \div 20v_0$	89	26.4
$4v \div 5v$	34	10.1	$20v_0 \div 25v_0$	33	9,8
$5v \div 6v$	28	8.3	$25v_0 \div 30v_0$	27	8.0
$6v \div 7v$	12	3.6	$30v_0 \div 35v_0$	9	2.7
$7v \div 8v$	2	0.6	$35v_0 \div 40v_0$	6	1.8
$8v \div 9v$	2	0.6	$40v_0 \div 45v_0$	3	0.9
$9v \div 10v$	0	0.0	$45v_0 \div 50v_0$	0	0.0

in the detection of more objective criterions for the differentiation of groups with an increased carcinogenic risk. From our point of view in vitro testing exposure of lymphocytes can be recommended as a methodical approach for evaluating chromosomal stability. The preliminary data allow us to conclude that low-dose radiation (exterior and interior) of clean-up workers is a factor which destabilises the human somatic cells genotype. Thus, the clean-up workers with increased individual radiosensitivity are referred to as a group of increased carcinogenic risk.

Conclusion

Cytogenetic examinations are necessary when monitoring the Chernobyl accident, they allow us to reveal groups with an increased cancerogenic risk.

Note

i - is the index number, α_i - is the left confidential limit for index x_i in group X, β_i - is the right confidential limit for index x_i in group X, α_i^* - is the left confidential limit for index x_i in group Y, β_i - is the right confidential limit for index x_i in group Y, a_i - is the length of the confidential interval for index x_i in group X, b_i - is the length of the confidential interval for index x_i in group Y, c_i - is the length of confidential intervals crossing for index x_i in groups X and Y, I_i - is the degree of x_i self descriptiveness index. Confidential intervals are designed by rule $2s_i$ with a significance value of 5%.

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